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Epidemiology of extended-spectrum ß-lactamase producing *Escherichia coli* in the human-livestock environment

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Abstract

The detection of extended-spectrum ß-lactamase (ESBL) producers in the fecal flora of healthy food-producing animals has increased in recent years. This is mainly attributed to the intense use of antibiotics in this sector. There is growing concern regarding the risk of spread of such bacteria, especially *Escherichia coli* and *Salmonella*, to humans and to the environment. The occurrence of ESBL producers in the major groups of livestock, i.e., poultry, pigs, cattle and sheep is highlighted and discussed with regard to data that provide evidence for transmission of their resistance traits from livestock to humans and to farm environments.

Keywords

Antimicrobial resistance; *E. coli*; animals; farmers; farm surroundings

Introduction

In human as in veterinary medicine, antimicrobial agents are used therapeutically for the treatment of specific conditions of bacterial etiology. Concerning modern livestock industry, large amounts of antimicrobial agents are additionally used for prophylactic and metaphylactic purposes, as well as for growth promotion. Although antibiotic resistance genes occur naturally in many in bacteria, overuse of antimicrobials and inappropriate antibiotic stewardship in both human and veterinary sectors have favored the emergence and dissemination of antimicrobial resistance (AMR) in bacteria. AMR has now become a public health problem of global dimensions. Major health organizations as well as governments acknowledge it as one the most significant threats not only to human health, but also to agriculture and economic growth [1-3]. In acknowledgement of this dire situation, an increasing number of governments have launched national and international strategies for tackling AMR, including USA, Canada, Great Britain, Germany and Australia [4-8]. The declared aim is to improve antimicrobial stewardship, monitoring and surveillance, international collaboration and, most recently in the case of the USA, reduce the use of antibiotics for growth promotion in food animals.

With regard to AMR, the US Centers for Disease Control and Prevention (CDC) prioritizes microorganisms into one of the three categories urgent, serious, and concerning [3]. Among the bacteria that are thereby categorized at a serious threat level, CDC lists extended-spectrum ß-lactamase (ESBL) producing Enterobacteriaceae. ESBL-producing bacteria are of particular concern, since they are resistant to many ß-lactam antibiotics including cephalosporins of the 3rd and 4th generation such as cefpodoxime, ceftazidime, cefotaxime and cefepime, all of which are labeled by the WHO as critically important antimicrobials for human medicine [9]. Beyond the use in human medicine, 3rd generation cephalosporins such as cefquinome and ceftiofur are important for treating infections in food-producing animals. The emergence of ESBL-producing Enterobacteriaceae in livestock and food of animal origin

has been documented in recent years and has raised concern regarding the risk of spread to humans [10].

This review addresses the epidemiology of ESBL-producing bacteria in the human-livestock environment and highlights recent studies that offer further insight into the mechanisms of putative interspecies transmission routes.

Overview of ESBLs

ESBLs are bacterial enzymes that hydrolyze the ß-lactam ring of extended-spectrum cephalosporins (ceftazidime or cefotaxime) and monobactams (aztreonam) [11]. They represent the most important mechanism of antibacterial resistance in Enterobacteriaceae, dramatically reducing the efficacy of modern expanded-spectrum cephalosporins (except cephamycins and carbapenems). Based on their primary sequence homology [12] and their substrate profiles [13], ESBLs can be categorized into classes and groups, respectively, whereby the majority of ESBLs belong to Ambler class A and to the Bush group 2be. Originally detected in human clinical isolates associated with nosocomial infections in the early 1990s, the plasmid-mediated TEM- and SHV-ESBLs were predominant over the following decade, but have since been replaced by the globally disseminated CTX-M ß-lactamases [14-16]. These are cefotaximases that originated from environmental *Kluyvera* spp through mobilization of chromosomal $bla_{\text{CTX-M}}$ genes into mobile genetic elements such as transposons and plasmids [17]. The CTX-M enzymes are classified according to their amino acid similarities into the five major groups CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9 and CTX-M-25 [18]. To date, more than 150 different CTX-M enzymes have been identified and registered in the Lahey database (www.lahey.org/studies/). The most prevalent ESBLs belong to CTX-M group 1, which includes the enzymes CTX-M-1 and CTX-M-15, and the group 9 enzyme CTX-M-14 [19].

Plasmids and mobile genetic elements play a key role in the dissemination of *bla*$_{ESBL}$ genes

Plasmids can be defined as double stranded circular DNA units that replicate autonomously within the bacterial cell and can be transferred via conjugation to bacteria of the same or of different species, promoting horizontal gene transfer [20]. Plasmids are categorized into families referred to as incompatibility (Inc) groups (or replicon types), and plasmid multilocus sequence types (pMLST), both of which can be determined using PCR methods [20, 21]. Different *bla*$_{ESBLs}$ are associated with particular plasmid replicon types. For example, *bla*$_{CTX\text{-}M\text{-}15}$ is frequently identified on IncFII plasmids, which is a narrow host range plasmid group well adapted to proliferate in *E. coli*, whilst *bla*$_{CTX\text{-}M\text{-}1}$ is predominantly found on plasmids belonging to broad host range IncI and IncN groups which are also found in isolates of animal origin [18,22]. In addition, many resistance plasmids harbor toxin-antitoxin (TA) factors, which are so-called addiction systems that eliminate any post-segregational daughter cells that have not inherited the plasmid. This allows for vertical gene transfer and stable maintenance of plasmids within the bacterial population, even without selective pressure [23, 24]. Addiction systems have been demonstrated for IncF plasmids harboring *bla*$_{CTX\text{-}M\text{-}15}$ [25] and for IncI plasmids harboring *bla*$_{CTX\text{-}M\text{-}1}$ [26]. The presence of addiction systems in plasmids carrying *bla*$_{ESBL}$ genes and other resistance determinants contribute to epidemiological success of ESBL-producing bacteria.

Clones are a further important driving force of the spread of *bla*$_{ESBL}$ genes

In clinical settings, CTX-M-15 has emerged as one of the most frequent ESBLs associated with a pandemic, highly virulent, multidrug resistant *E.coli* defined by phylogenetic grouping [27] and multilocus sequence typing (MLST) [28], as clone *E. coli* B2:ST131. This clone is considered to contribute greatly to the global dissemination of CTXM-15 and is associated with hospital as well as community-acquired infections [29, 30]. Other multidrug resistant

enterobacterial lineages of clinical importance include virulent extraintestinal clones belonging to phylogenetic group D: *E. coli* D:ST38, *E. coli* D:ST405 and *E. coli* D:ST648 are all associated with CTX-M-9, CTX-M-14 and CTX-M-15 [31, 32].

These and other clones and strains may harbor genes that code for virulence factors (VF) that are thought to increase adaptability, competitiveness, and ability to efficiently colonize the gastrointestinal tract and contribute to the successful dissemination of the clone [32].

ESBLs beyond the clinical setting

Colonization of the gastrointestinal tract is an important factor regarding the epidemiology of ESBL-producing Enterobacteriaceae. Recent years have seen a number of studies dedicated to screening for ESBL-producing Enterobacteriaceae in the fecal flora of humans in community settings, in animals and the environment. Fecal carriage of CTX-M- ESBLs in healthy humans is increasing significantly worldwide. A recent analysis of this trend detected vast differences between the world regions: In Europe, carriage rates range around or below 10% whereas in parts of Southeast Asia and the Eastern Mediterranean over 50% of the population is currently found to harbor CTX-M- producing *E. coli*. In most regions, CTX-M-15 is the most prevalent ESBL, and is increasingly replacing CTX-M-14 in Asia, as well as CTX-M-2 in South America [33]. Other ESBL variants include CTX-M-1 and SHV-12 [34]. The increasing prevalence raises questions concerning the risk factors of gut colonization among healthy people. Consumption of antimicrobials, hospitalization, premorbidity, African or Asian family background and international travel have all have been established as risk factors for an individual to become colonized with ESBL-producing bacteria [35-40]. Moreover, there is growing concern that resistant *E coli* originating from food animals may present a reservoir of ESBL-producing bacteria and an important threat to human health. Several studies have drawn attention to the possibility of the transmission of bla_{ESBL} genes, their encoding plasmids and the *E. coli* isolates via fresh food, in particular from poultry meat

[41-44]. From such studies it has become evident that CTX-M-1 is currently one of the most prevalent ESBL in food-producing animals, at least in poultry. Accordingly, CTX-M-1 constitutes the most frequently reported ESBL variant in the EU in *E. coli* originating from food-producing animals and foods [45]. It is becoming increasingly clear that livestock represents a major reservoir of ESBL-producing *E. coli,* although the actual impact of this reservoir on human health is still insufficiently understood, and linking antibiotic use in food-producing animals with resistance in human isolates is discussed controversially. Thus, understanding epidemiology of extended-spectrum ß-lactamase (ESBL)-producing Enterobacteriaceae in the human-livestock environment is important. Recent studies that provide further insight into this matter are listed in Table 1 and discussed below.

ESBLs in livestock

Poultry

As stated above, poultry represents a major reservoir of CTX-M-1-producing *E. coli.* Zurfluh et al. [46] found that *E. coli* harboring $bla_{\text{CTX-M-1}}$ on highly similar plasmids belonging to group IncI1 and pMLST 3 (IncI1/ST3 plasmids), are persistent at all levels in the Swiss broiler production farms, including day-old chicks. From this and other investigations in the Netherlands and Germany [47,48], it has become evident that the hatching eggs at the tip of the production pyramid play a central role as an entry point of ESBL-producing *E. coli* into broiler farms. In a longitudinal study, Laube and colleagues [48] demonstrated that ESBL- producing *E. coli* increase over time in broilers during the fattening period. It has been shown in all studies mentioned above that CTX-M-1-producing *E. coli* are maintained in broiler farms at detection levels between 50% and 100%, even in the absence of antimicrobial selective pressure [49]. This high rate of contamination raises questions regarding the risks that occupationally involved humans and their environment are exposed to. An investigation of Dierikx and collaborators [50] (Table 1) revealed that the prevalence of ESBL-producers in

Dutch broiler farmers was 33%, which is much higher compared to the 4.9% described previously for the Dutch population [51]. In *E. coli* isolates from fecal samples from five of the six farmers, the combination of detected ESBL genes ($bla_{\text{CTX-M-1}}$ and $bla_{\text{SHV-12}}$) and the plasmid group on which the genes were located (IncI1 and IncN, respectively), corresponded to genes and plasmids found in isolates from cloacal samples from the broilers. Huijbers et al. [52] (Table 1) provided evidence of clonal spread of ESBL-producing *E. coli*, including clones A:ST10(CTX-M-1), A:ST23(CTX-M-1) and extraintestinal pathogenic clone D:ST648(SHV-12), between broilers and farm workers and established physical contact with live broilers as a risk factor for fecal carriage of ESBL-producers in humans. Carriage rates for individuals with close contact to live broilers were found to be 27.1%. For family members and employees living on the farm but with no direct contact to live broilers it was 14.3%, indicating human-to-human transmission. Interestingly, the risk for people living in areas around broiler farms was previously found not to be greater than for the general population [53].

In longitudinal studies investigating the transmission pathways of ESBL-producing *E. coli* from seven German broiler chicken farms and the farms' environs, Laube et al. [54] (Table 1) analyzed samples taken from chicken feces, air and dust inside broiler barns and slurry, air, meadows, fields, grass, forest floors and roads surrounding the broiler farms. It was thereby demonstrated that ESBL-producing *E. coli* can be transmitted via the ventilation system to a distance of at least 50 m from farms. Other emissions included the application of slurry from the broiler farms to fields. On Dutch broiler farms and on laying farms Blaak and collaborators [55] (Table 1) found that soil, barn rinse water, flies and fecal samples of farm resident pets tested positive for ESBL-producers. With regard to ST, phylogenetic group and ESBL-genotype, 60% of these isolates were identical to isolates from poultry feces at the same farms. Besides CTX-M-1, the ESBLs SHV-12 and TEM-52 were frequently observed (Table 1). These findings are depicted schematically in Fig. 1. Flies circulating throughout

broiler farms, mainly *Musca domestica*, have been implicated as vectors of dissemination of ESBL-producers [56] (Table 1). Furthermore, in this study, Solà-Ginés and co-workers detected genes encoding virulence factors, predominantly in *E. coli* A:ST10(CTX-M-1), including *fimH*, *astA*, *iutA*, *iroN*, *iss*, *traT*, all of which are genes involved in avian disease as well as human extraintestinal infections, such as urinary tract infections [57]. The observations from the studies outlined above suggest a considerable risk of diffusion of ESBL-producing *E. coli* clones with possible zoonotic potential from broiler farms.

Pigs

Frequently observed ESBL variants in *E. coli* from fecal samples of healthy pigs include SHV-12, CTX-M-1-, CTX-M-9 and CTX-M-14 and CTX-M-15. [58,59] (Table 1). Recently, pig farms have been identified as reservoirs for TEM-52 [60] (Table 1). TEM-52 was found associated to the most part with IncI1/ST3 plasmids and epidemic clone *E. coli* A:ST10. Additionally, CTX-M-1 and CTX-M-32 within IncN/ST1 plasmids and epidemic clone *E. coli* A:ST10 were detected in feces and on hides of pigs, in pig feed and in liquid manure on pig farms located throughout Portugal [60].

Fecal carriage of ESBL-producing *Enterobacteriaceae* in pigs is a dynamic process, as outlined in a longitudinal study by Hansen and collaborators [61] (Table 1). This Danish investigation analysed the fecal flora of pigs bred in farrow-to-finish productions systems. In such systems, the sows, piglets, weaners and finishers are housed in separated sections on the same farm. On all farms, it was shown that fecal carriage of CTX-M-producers was highest in the farrowing area and coincided with the use of extended-spectrum penicillins in sows and farrows. However, fecal carriage decreased significantly after weaning and was lowest in finishers just before slaughter, suggesting that certain practices during the process of production influence the status of fecal carriage. One such process was found to be thorough cleaning and disinfection of housing sections prior to moving batches of pigs. Thus, although

the farrowing area may serve as a reservoir of ESBL-producers, the contamination of following batches can be reduced by good sanitation.

Hammerum and co-workers [62] (Table 1) showed that farm workers with direct contact to ESBL-positive pigs had a fecal carriage rate of ESBL-producers of 21%. On four of 20 farms, the combination of ESBL genes (mainly CTX-M-1 encoded on IncI1, IncF or IncN plasmids) and ESBL-producing *E. coli* clones, notably *E. coli* A:ST10(CTX-M-1) and A:ST10(CTX-M-14), were shared with fecal isolates from the pigs on the same farm (Fig. 1). This strongly suggests transmission between pigs and humans. Interestingly, transfer of ESBL-producing *E. coli* from farmers working with pigs to family members not working with pigs was not detected.

Similar to broiler farms, emissions of ESBL producers from pig farms into the environment have been reported: An investigation conducted by von Salviati and colleagues [63] (Table 1) on seven German pig fattening farms detected identical ESBL-*E. coli* isolates (with CTX-M-1 representing the majority) in pig feces, dust, barn air, flies and mouse droppings, and further, in slurry and in digestate from biogas plants, both of which were intended for application on fields as fertilizers. In addition, fields fertilized one to seven months before sampling tested positive for ESBL-producers. Reviewing the results of this systematic study, the authors conclude that slurry from pig farms represents a significant emission source for ESBL-producers. Furthermore, they suggest that biogas plants which apply mesophilic digestion, i.e., between 20°C and 40°C, may need to improve treatment conditions to reduce the bacterial load in the digestate.

In pigs, respiratory infections are the main indication for using antimicrobials, followed by intestinal diseases in piglets, weaners and fattening pigs and diseases of reproductive organs in sows [64]. In a study on the impact of ceftiofur treatment of pigs, Fleury et al. [65] observed a dramatic increase of shedding of CTX-M-1 *E. coli* for three days after injection.

Similar results were obtained by Vasseur and colleagues [66], who showed that therapeutic doses of cefquinome resulted in a massive amplification of CTX-M-1 producing *E. coli* in the guts of laboratory rats.

Cattle and sheep

ESBLs in cattle were first observed in fecal samples of cattle and cattle carcasses in Japan in 2000 [67]. The emergence of CTX-M-2 in cattle was attributed to the use of ceftiofur, which was the only expanded-spectrum cephalosporin approved for livestock in Japan at that time. Since then, CTX-M-1, CTX-M-13 and CTX-M-14 have been detected sporadically in *E. coli* from cattle in Europe and Asia [10]. In 2010, CTX-M-1 producing *E. coli* was reported for the first time in cattle in the USA [68]. Currently, in Europe, the prevalence of ESBL-producers in fecal swabs of healthy cattle is 5%-13%, with CTX-M-1 the most prevalent variant [69,70]. Since 3rd generation cephalosporins, namely ceftiofur and cefquinome are frequently used to treat diseases such as pulmonary infections and mastitis in cattle, the question is controversially discussed of whether the therapeutic use of cephalosporins as well as the subtherapeutic use of other antimicrobials (i.e., growth promoters/digestive enhancers) has contributed to the emergence of ESBLs in cattle and other livestock. Schmid et al, [71] found that on German mixed dairy cattle/beef farms, ESBL-producing *E. coli* strains (mainly CTX-M-1-producers) were significantly more often detected in fecal samples from calves treated with antimicrobials (ß-lactams as well as others) than in untreated calves. They further stated that that calves being fed waste milk (which may contain antibiotic residues) harbored more ESBL-producing *E. coli*. Milk from cows with mastitis may contain CTX-M-1 and CTX-M-14 producing Enterobacteriaceae, albeit at a very low rate of 0.4%, as demonstrated in an extensive screening study conducted in France by Dahmen and co-workers [72].

Cottell and co-workers [73] (Table 1) observed the emergence of CTX-M-32 producing *E. coli* in the fecal samples of 29 of 88 (33%) steers receiving either ceftiofur (14 animals), chlortetracycline (4 animals), or both treatments (11 animals).

A longitudinal study conducted by Hordijk and collaborators [74] (Table 1) showed that veal calves collected from different EU countries to be distributed on Dutch fattening farms brought with them a high diversity of fecal ESBL-producers, with CTX-M-1, CTX-M-15, CTX-M-32 and TEM-52 detected most frequently. On all three fattening farms under observation, ESBL diversity declined after prophylactic antimicrobial group treatment, and was replaced over a time period of 6 weeks with farm-specific clones: *E. coli* ST57 harboring $bla_{\text{CTX-M-14}}$ on an IncF F2:A-:B- plasmid on one farm, and *E. coli* ST10 harboring CTX-M-14 on an IncK plasmid on another. The observations from this study suggest that decontaminating pens and other housing facilities prior to arrival of veal calves for fattening could help control (re)colonization of calves and the clonal spread of ESBL producers in veal calves before slaughter.

Currently, there is a paucity of data available on the fecal carriage rate and risk factors for individuals who have frequent contact with cattle. However, based on the studies on fecal carriage of poultry and pig farmers discussed above, it is reasonable to assume that a similar occupational hazard exists for cattle farmers. Moreover, pasture soil, manure from cattle and amended fields testing positive for ESBL-producing *E. coli* provide evidence for cattle farms as emission sources of ESBL producers [69] (Table 1, Fig. 1).

Reports on ESBL producers in fecal samples of sheep are rare, but show that to date CTX-M-14 predominates, at least in Switzerland [70] and the UK [75]. However, a recent study conducted by Ramos and collaborators [76] (Table 1) showed that CTX-M-32 is predominant in the feces of healthy sheep at slaughter in Portugal. Furthermore, this study revealed that ESBL producers isolated from cattle and sheep may contain genes encoding virulence factors such as *aer* and *fimA*, which are associated with extraintestinal pathogenic *E. coli*.

Together with other studies that analyze ESBL- producing *E. coli* in pigs and pork [59] and poultry and poultry meat [41], these results demonstrate that livestock may represent a reservoir of virulence genes as well as bla_{ESBLs}.

Pathogenic ESBL producers in livestock

Although livestock represent a major source of ESBL-producing commensal *E. coli*, it is unclear to what extent bla_{ESBL} genes are transferred to zoonotic pathogens. For example, only few ESBL-producing enterohaemorrhagic *E. coli* (EHEC) strains have been reported [77]. This is surprising, since cattle are recognized as the main reservoir of EHEC. Nevertheless, CTX-M-1 is detected increasingly in other zoonotic pathogens, mostly *Salmonella* spp, for instance in *S.* Anantum and *S.* Infantis from cattle and S. Paratyphi B from chicken in Germany [78], in *S.* Llandoff from chicken in France [79], in *Salmonella* spp. from turkeys and from a pig in USA [80], and in Salmonella 4,5,12:i:2- as well as in *S.* Bovismorbificans from pigs in the UK [81]. In all these cases, $bla_{\text{CTX-M-1}}$ was frequently harbored on IncI1 or IncN plasmids, which is suggestive of horizontal genetic transfer from commensal *E. coli* to Salmonella within the animal gut. Although currently rare, the prevalence of pathogenic CTX-M-1 producers of animal origin involved in human infections must therefore be expected to increase in future.

Conclusions

ESBL producers are frequent in the fecal flora of healthy livestock. Certain ESBLS (predominantly CTX-M-1, but also CTX-M- 14 and CTX-M-32) are characteristic to livestock and their occurrence is frequently linked to specific plasmids (IncI1, IncN) and *E. coli* clones (e.g., A:ST10, A:ST23). Data from recent studies suggest that the therapeutic use of expanded-spectrum ß-lactams, in particular ceftiofur in cattle and pigs promotes the

emergence and dissemination of CTX-M-1 and CTX-M-32 producing *E. coli* within farms. The studies highlighted in this review provide evidence for the transmission of ESBL-producing commensal *E. coli* from livestock to humans occupationally involved in farming and to the environment of livestock farms, including air, pathways, slurry, fertilized fields and digestate from biogas plants. The impact of ESBL producers of animal origin on human health and environmental integrity is just beginning to become apparent. The implementation of international and national mitigation strategies including increased surveillance and monitoring of AMR as well as the optimization of therapeutic strategies for livestock are necessary. On regional and local scales, it is urgent to provide solution-focused approaches. These could include improvements in biosecurity and hygiene practices on farms to reduce the occupational risk for farmers, and new approaches targeting the safer disposal of gastrointestinal waste material (slurry, manure, biogas digestate) in order to limit the transmission of ESBL producing *E. coli* into the environment.

References

Recently published papers of particular interest have been highlighted as •

1. World Health Organization WHO. Antimicrobial Resistance: global report on surveillance 2014. Available from http://www.who.int/drugresistance/documents/surveillancereport/en.
2. International Monetary Fund. The fight for global health. FINANCE and DEVELOPMENT 2014. Available at http://www.imf.org/external/pubs/ft/fandd/2014/12/pdf/fd1214.pdf.
3. Centers for Disease Control and Prevention CDC. Antibiotic resistance threats in the United States, 2013. Available at http://www.cdc.gov/drugresistance/threat-report-2013/.
4. President's Counsil of Advisors on Science and Technology. Antibiotic resistance working group. Report to the president on combating antibiotic resistance. 2014. Available at www.whitehouse.gov/ostp/pcast.
5. Public Health Agency of Canada. Antimicrobial resistance and use in Canada: A federal framework for action. 2014. Available at http://healthycanadians.gc.ca/drugs-products-medicaments-produits/antibiotic-resistance-antibiotique/antimicrobial-framework-cadre-antimicrobiens-eng.php.
6. HM Government. UK 5 year antimicrobial resistance (AMR) strategy 2013–2018. Annual progress report and implementation plan, 2014. Available at www.gov.uk/government/publications.
7. Federal Government of Germany. Deutsche Antibiotika-Resistenzstrategie [German antibiotic resistance strategy] DART 2020. Antibiotika-Resistenzen bekämpfen zum Wohl von Mensch und Tier [Combating antibiotic resistance for the benefit of human and animal health]. 2015. Available at http://www.bmel.de/SharedDocs/Downloads/Broschueren/DART2020.pdf?__blob=publicationFile.

8. Australian Government Department of Health and Department of Agriculture. National antimicrobial resistance strategy 2015-2019. Responding to the threat of antimicrobial resistance. 2015. Online ISBN: 978-1-76007-192-9.

9. World Health Organization WHO. Critically important antimicrobials for human medicine - 3rd rev. 2011. Available at http://www.who.int/foodborne_disease/resistance/agisar/en/.

10. Carattoli A. Animal reservoirs for extended spectrum beta-lactamase producers. Clin Microbiol Infect. 2008;14:117-23.

11. Matagne A, Lamotte-Brasseur J, Frère JM. Catalytic properties of class A beta-lactamases: Efficiency and diversity. Biochem J. 1998;330:581-98.

12. Ambler RP, Coulson AF, Frère JM, Ghuysen JM, Joris B, Forsman M, Levesque R C, Tiraby G, Waley S G. A standard numbering scheme for the class A beta-lactamases. Biochem J. 1991;276:269-72.

13. Bush K, Jacoby GA. Updated functional classification of beta-lactamases. Antimicrob Agents Chemother. 2010;54:969-76.

14. Paterson DL, Bonomo RA. Extended-spectrum β-lactamases: A clinical update. Clin Microbiol Rev 2005;18:657-86.

15. Perilli M, Dell'Amico E, Segatore B, de Massis MR, Bianchi C, Luzzaro F, Rossolini GM, Toniolo A, Nicoletti G, Amicosante G. Molecular characterization of extended-spectrum beta-lactamases produced by nosocomial isolates of Enterobacteriaceae from an Italian nationwide survey. J Clin Microbiol. 2002;40:611-4.

16. Jacoby GA, Luisa Silvia Munoz-Price MD. The new ß-lactamases. N Engl J Med. 2005; 352:380-91.

17. Cantón R, González-Alba JM, Galán JC. CTX-M enzymes: Origin and diffusion. Front Microbiol. 2012;3:110.

18. Naseer U, Sundsfjord A. The CTX-M conundrum: Dissemination of plasmids and *Escherichia coli* clones. Microb Drug Resist. 2011;17:83-97.

19. Bush K. Proliferation and significance of clinically relevant β-lactamases. Ann N Y Acad Sci. 2013;1277:84-90.

20. Carattoli A. Resistance plasmid families in Enterobacteriaceae. Antimicrob Agents Chemother. 2009;53:2227-38.

21. Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, Threlfall EJ. Identification of plasmids by PCR-based replicon typing. J Microbiol Meth. 2005;63:219-28.

22. Johnson TJ, Wannemuehler YM, Johnson SJ, Logue CM, White DG, Doetkott C, Nolan LK. Plasmid replicon typing of commensal and pathogenic *Escherichia coli* isolates. Appl Environ Microbiol. 2007;73:1976-83.

23. Van Melderen L, Saavedra De Bast M. Bacterial toxin-antitoxin systems: More than selfish entities? PLoS Genet. 2009;5:e1000437.

24. Hayes F. Toxins-antitoxins: Plasmid maintenance, programmed cell death, and cell cycle arrest. Science. 2003;301:1496-9.

25. Mnif B, Harhour H, Jdidi J, Mahjoubi F, Genel N, Arlet G, Hammami A. Molecular epidemiology of extended-spectrum beta-lactamase-producing *Escherichia coli* in Tunisia and characterization of their virulence factors and plasmid addiction systems. BMC Microbiol. 2013;13:147.

26. Wang J, Stephan R, Zurfluh K, Hächler H, Fanning S. Characterization of the genetic environment of bla_{ESBL} genes, integrons and toxin-antitoxin systems identified on large transferrable plasmids in multi-drug resistant *Escherichia coli*. Front Microbiol 2015;5:716.

27. Clermont O, Bonacorsi S, Bingen E. Rapid and simple determination of the *Escherichia coli* phylogenetic group. Appl Environ Microbiol 2000;66:4555-8.

28. Wirth T, Falush D, Lan R, Colles F, Mensa P, Wieler LH, Karch H, Reeves PR, Maiden MC, Ochman H, Achtman M. Sex and virulence in *Escherichia coli*: An evolutionary perspective. Mol Microbiol 2006;60:1136-51.

29. Singer RS. Urinary tract infections attributed to diverse ExPEC strains in food animals: Evidence and data gaps. Front Microbiol. 2015;6:28.

30. Rogers BA, Sidjabat HE, Paterson DL. *Escherichia coli* o25b-st131: A pandemic, multiresistant, community-associated strain. J Antimicrob Chemother. 2011;66:1-14.

31. D'Andrea MM, Arena F, Pallecchi L, Rossolini GM. CTX-M-type β-lactamases: A successful story of antibiotic resistance. Int J Med Microbiol. 2013;303:305-17.

32. Van der Bij AK, Peirano G, Pitondo-Silva A, Pitout JD. The presence of genes encoding for different virulence factors in clonally related *Escherichia coli* that produce CTX-Ms. Diagn Microbiol Infect Dis. 2012;72:297-302.

33. Woerther PL, Burdet C, Chachaty E, Andremont A. Trends in human fecal carriage of extended-spectrum β-lactamases in the community: Toward the globalization of CTX-M. Clin Microbiol Rev. 2013;26:744-58.

34. Geser N, Stephan R, Korczak BM, Beutin L, Hächler H. Molecular identification of extended-spectrum-β-lactamase genes from Enterobacteriaceae isolated from healthy human carriers in Switzerland. Antimicrob Agents Chemother. 2012;56:1609-12.

35. Nüesch-Inderbinen MT, Abgottspon H, Zurfluh K, Nüesch HJ, Stephan R, Hächler H. Cross-Sectional study on fecal carriage of Enterobacteriaceae with resistance to extended-spectrum cephalosporins in primary care patients. Microb Drug Resist. 2013;19:362-69.

36. Nicolas-Chanoine MH, Jarlier V, Robert J, Arlet G, Drieux L, Leflon-Guibout V, Laouénan C, Larroque B, Caro V, Mentré F, Group Coli β. Patient's origin and lifestyle associated with CTX-M-producing *Escherichia coli*: A case-control-control study. PLoS One. 2012;7(1):e30498.

37. Leistner R, Meyer E, Gastmeier P, Pfeifer Y, Eller C, Dem P, Schwab F. Risk factors associated with the community-acquired colonization of extended-spectrum beta-lactamase (ESBL) positive *Escherichia coli*. An exploratory case-control study. PLoS One. 2013;8(9):e74323.

38. Wickramasinghe NH, Xu L, Eustace A, Shabir S, Saluja T, Hawkey PM. High community faecal carriage rates of CTX-M ESBL-producing *Escherichia coli* in a specific population group in Birmingham, UK. J Antimicrob Chemother. 2012; 67:1108-13.

39. Kantele A, Lääveri T, Mero S, Vilkman K, Pakkanen SH, Ollgren J, Antikainen J, Kirveskari J. Antimicrobials increase travelers' risk of colonization by extended-spectrum betalactamase-producing Enterobacteriaceae. Clin Infect Dis. 2015; doi: 10.1093/cid/ciu957.

40. Tängdén T, Cars O, Melhus A, Löwdin E. Foreign travel is a major risk factor for colonization with *Escherichia coli* producing ctx-m-type extended-spectrum beta-lactamases: A prospective study with swedish volunteers. Antimicrob Agents Chemother. 2010; 54:3564-8.

41. Leverstein-van Hall MA, Dierikx CM, Cohen Stuart J, Voets GM, van den Munckhof MP, van Essen-Zandbergen A, Platteel T, Fluit AC, van de Sande-Bruinsma N, Scharinga J, Bonten MJ, Mevius DJ, National ESBL surveillance group. Dutch patients, retail chicken meat and poultry share the same ESBL genes, plasmids and strains. Clin Microbiol Infect. 2011;17:873-80.

42. Abgottspon H, Stephan R, Bagutti C, Brodmann P, Hächler H, Zurfluh K. Characteristics of extended-spectrum cephalosporin-resistant *Escherichia coli* isolated from Swiss and imported poultry meat. J Food Prot. 2014;77:112-5.

43. Stewardson AJ, Renzi G, Maury N, Vaudaux C, Brossier C, Fritsch E, Pittet D, Heck M, van der Zwaluw K, Reuland EA, van de Laar T, Snelders E, Vandenbroucke-Grauls C, Kluytmans J, Edder P, Schrenzel J, Harbarth S. Extended-Spectrum β-lactamase-producing Enterobacteriaceae in hospital food: A risk assessment. Infect Control Hosp Epidemiol. 2014;35:375-83.

44. Cohen Stuart J, van den Munckhof T, Voets G, Scharringa J, Fluit A, Hall ML. Comparison of ESBL contamination in organic and conventional retail chicken meat. Int J Food Microbiol. 2012; 154:212-4.

45. European Food Safety Authority EFSA. Scientific opinion on the public health risks of bacterial strains producing extended-spectrum β-lactamases and/or AmpC β-lactamases in food and food-producing animals. EFSA J 2011; doi:10.2903/j.efsa.2011.2322.

46. Zurfluh K, Wang J, Klumpp J, Nüesch-Inderbinen M, Fanning S, Stephan R. Vertical transmission of highly similar *bla*$_{\text{CTX-M-1}}$-harboring IncI1 plasmids in *Escherichia coli* with different MLST types in the poultry production pyramid. Front Microbiol. 2014; doi: 10.3389/fmicb.2014.00519.

47. Dierikx CM, van der Goot JA, Smith HE, Kant A, Mevius DJ. Presence of ESBL/AmpC-producing *Escherichia coli* in the broiler production pyramid: A descriptive study. PLoS One. 2013;8(11):e79005.

48. Laube H, Friese A, von Salviati C, Guerra B, Käsbohrer A, Kreienbrock L, Roesler U. Longitudinal monitoring of extended-spectrum-beta-lactamase/ampc-producing *Escherichia coli* at german broiler chicken fattening farms. Appl Environ Microbiol. 2013;79:4815-20.

49. Bortolaia V, Guardabassi L, Bisgaard M, Larsen J, Bojesen AM. *Escherichia coli* producing CTX-M-1,-2, and-9 group β-lactamases in organic chicken egg production. Antimicrob Agents Chemother. 2010;54:3527-8.

50.•Dierikx C, van der Goot J, Fabri T, van Essen-Zandbergen A, Smith H, Mevius D. Extended-spectrum-β-lactamase- and AmpC-β-lactamase-producing *Escherichia coli* in Dutch broilers and broiler farmers. J Antimicrob Chemother 2013, Jan;68(1):60-7.

Demonstrate that broiler farmers are at higher risk than the general population of fecal carriage of ESBL producing *E. coli*.

51. Overdevest I, Willemsen I, Rijnsburger M, Eustace A, Xu L, Hawkey P, Heck M, Savelkoul P, Vandenbroucke-Grauls C, van der Zwaluw K. Extended-spectrum β-lactamase genes of *Escherichia coli* in chicken meat and humans, the Netherlands. Emerg Infect Dis. 2011;17:1216-22.

52.•Huijbers PM, Graat EA, Haenen AP, van Santen MG, van Essen-Zandbergen A, Mevius DJ, van Duijkeren E, van Hoek AH. Extended-spectrum and AmpC β-lactamase-producing *Escherichia coli* in broilers and people living and/or working on broiler farms: Prevalence, risk factors and molecular characteristics. J Antimicrob Chemother. 2014;69:2669-75. **Provide evidence for clonal transmission of ESBL-producers from broilers to humans and from humans to humans on broiler farms.**

53. Huijbers PMC, Kraker DM, Graat EAM, Hoek A, Santen MG, Jong DM, Duijkeren VE, Greeff DS. Prevalence of extended-spectrum β-lactamase-producing Enterobacteriaceae in humans living in municipalities with high and low broiler density. Clin Microbiol Inf. 2013;19:256-9.

54.•Laube H, Friese A, von Salviati C, Guerra B, Rösler U. Transmission of ESBL/AmpC-producing *Escherichia coli* from broiler chicken farms to surrounding areas. Vet Microbiol. 2014;172:519-27. **Systematic investigation that shows that ESBL-producers are transmitted from broiler farms to the environment via stable airing systems and contaminated slurry used for the fertilization of fields.**

55. Blaak H, van Hoek AH, Hamidjaja RA, van der Plaats RQ, Kerkhof-de Heer L, de Roda Husman AM, Schets FM. Distribution, numbers, and diversity of ESBL-producing *E. coli* in the poultry farm environment. PLoS One. 2015;10(8):e0135402.

56. Solà-Ginés M, González-López JJ, Cameron-Veas K, Piedra-Carrasco N, Cerdà-Cuéllar M, Migura-Garcia L. Houseflies (*Musca domestica*) as vectors for extended-spectrum β-lactamase-producing *Escherichia coli* on Spanish broiler farms. Appl Environ Microbiol. 2015;81:3604-11.

57. Maluta RP, Logue CM, Casas MR, Meng T, Guastalli EA, Rojas TC, Montelli AC, Sadatsune T, de Carvalho Ramos M, Nolan LK, da Silveira WD. Overlapped sequence types (STs) and serogroups of avian pathogenic (APEC) and human extra-intestinal pathogenic (ExPEC) *Escherichia coli* isolated in Brazil. PLoS One. 2014;9(8):e105016.

58. dos Santos L, Moura RA, Ramires PA, Pestana de Castro A, Lincopan N. Current status of extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae in animals. In: Méndez-Vilas A, editor. Microbial pathogens and strategies for combating them: Science, technology and education. Formatex Research Center, Badajoz 2013. pp. 1600-7.

59. Hammerum AM, Jakobsen L, Olsen SS, Agersø Y. Characterization of CTX-M-14-and CTX-M-15-producing *Escherichia coli* of porcine origin. J Antimicrob Chemother. 2012;67:2047-9.

60. Rodrigues C, Machado E, Peixe L, Novais A. IncI1/ST3 and IncN/ST1 plasmids drive the spread of $bla_{\text{TEM-52}}$ and $bla_{\text{CTX-M-1/-32}}$ in diverse *Escherichia coli* clones from different piggeries. J Antimicrob Chemother. 2013;68:2245-8.

61. Hansen KH, Damborg P, Andreasen M, Nielsen SS, Guardabassi L. Carriage and fecal counts of CTX-M-producing *Escherichia coli* in pigs: A longitudinal study. Appl Environ Microbiol. 2013;79:794-8.

62.•Hammerum AM, Larsen J, Andersen VD, Lester CH, Skytte TSS, Hansen F, Olsen SS, Mordhorst H, Skov RL, Aarestrup FM. Characterization of extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* obtained from Danish pigs, pig farmers and their families from farms with high or no consumption of third-or fourth-generation cephalosporins. J Antimicrob Chemother. 2014;69:2650-7. **Provides evidence for transfer of ESBL-producing E. coli and plasmids between pigs and pig farmers.**

63.•von Salviati C, Laube H, Guerra B, Roesler U, Friese A. Emission of ESBL/AmpC-producing *Escherichia coli* from pig fattening farms to surrounding areas. Vet Microbiol. 2015;175:77-84. **Identification of airing systems, slurry and digestate from biogas**

plants as emission sources for ESBL-producing *E. coli* from pig farms to their surroundings.

64. van Rennings L, von Münchhausen C, Ottilie H, Hartmann M, Merle R, Honscha W, Käsbohrer A, Kreienbrock L. Cross-sectional study on antibiotic usage in pigs in Germany. PLoS One 2015;10(3):e0119114.

65. Fleury MA, Mourand G, Jouy E, Touzain F, Le Devendec L, de Boisseson C, Eono F, Cariolet R, Guérin A, Le Goff O, Blanquet-Diot S, Alric M, Kempf I. Impact of ceftiofur injection on gut microbiota and *Escherichia coli* resistance in pigs. Antimicrob Agents Chemother. 2015;59:5171-80.

66. Vasseur MV, Laurentie M, Rolland J-G, Perrin-Guyomard A, Henri J, Ferran AA, Toutain P-L, Bousquet-Mélou A. Low or high doses of cefquinome targeting low or high bacterial inocula cure *Klebsiella pneumoniae* lung infections but differentially impact the levels of antibiotic resistance in fecal flora. Antimicrob Agents Chemother. 2014;58:1744-8.

67. Shiraki Y, Shibata N, Doi Y, Arakawa Y. *Escherichia coli* producing CTX-M-2 beta-lactamase in cattle, Japan. Emerg Infect Dis. 2004;10:69-75.

68. Wittum TE, Mollenkopf DF, Daniels JB, Parkinson AE, Mathews JL, Fry PR, Abley MJ, Gebreyes WA. CTX-M-type extended-spectrum β-lactamases present in *Escherichia coli* from the feces of cattle in Ohio, United States. Foodborne Pathog Dis. 2010, Dec;7:1575-9.

69. Hartmann A, Locatelli A, Amoureux L, Depret G, Jolivet C, Gueneau E, Neuwirth C. Occurrence of CTX-M producing *Escherichia coli* in soils, cattle, and farm environment in france (Burgundy region). Front Microbiol. 2012; doi: 10.3389/fmicb.2012.00083.

70. Geser N, Stephan R, Hächler H. Occurrence and characteristics of extended-spectrum β-lactamase (ESBL) producing Enterobacteriaceae in food producing animals, minced meat and raw milk. BMC Vet Res 2012; doi:10.1186/1746-6148-8-21.

71. Schmid A, Hörmansdorfer S, Messelhäusser U, Käsbohrer A, Sauter-Louis C, Mansfeld R. Prevalence of extended-spectrum β-lactamase-producing *Escherichia coli* on Bavarian dairy and beef cattle farms. Appl Environ Microbiol. 2013;79:3027-32.

72. Dahmen S, Métayer V, Gay E, Madec JY, Haenni M. Characterization of extended-spectrum beta-lactamase (ESBL)-carrying plasmids and clones of Enterobacteriaceae causing cattle mastitis in France. Vet Microbiol. 2013;162:793-9.

73. Cottell JL, Kanwar N, Castillo-Courtade L, Chalmers G, Scott HM, Norby B, Loneragan GH, Boerlin P. *bla*$_{\text{CTX-M-32}}$ on an IncN plasmid in *Escherichia coli* from beef cattle in the United States. Antimicrob Agents Chemother. 2013;57:1096-7.

74. Hordijk J, Mevius DJ, Kant A, Bos ME, Graveland H, Bosman AB, Hartskeerl CM, Heederik DJ, Wagenaar JA. Within-farm dynamics of ESBL/AmpC-producing *Escherichia coli* in veal calves: A longitudinal approach. J Antimicrob Chemother. 2013;68:2468-76.

75. Snow LC, Wearing H, Stephenson B, Teale CJ, Coldham NG. Investigation of the presence of ESBL-producing *Escherichia coli* in the North Wales and West Midlands areas of the UK in 2007 to 2008 using scanning surveillance. Vet Rec. 2011; doi: 10.1136/vr.100037.

76. Ramos S, Igrejas G, Silva N, Jones-Dias D, Capelo-Martinez J-L, Caniça M, Poeta P. First report of CTX-M producing *Escherichia coli*, including the new ST2526, isolated from beef cattle and sheep in Portugal. Food Control 2013;3:208-10.

77. Ewers, C. Extended-Spectrum β-Lactamase and AmpC β -lactamase-producing bacteria in livestock. In: Sing A, editor. Animals zoonoses-infections affecting humans and animals. Dordrecht: Springer Science and Business Media; 2015. pp. 379-406.

78. Rodríguez I, Barownick W, Helmuth R, Mendoza MC, Rodicio MR, Schroeter A, Guerra B. Extended-spectrum beta-lactamases and AmpC beta-lactamases in ceftiofur-resistant

Salmonella enterica isolates from food and livestock obtained in Germany during 2003-07. J Antimicrob Chemother. 2009;64:301-9.

79. Cloeckaert A, Praud K, Lefevre M, Doublet B, Pardos M, Granier SA, Brisabois A, Weill FX. IncI1 plasmid carrying extended-spectrum-beta-lactamase gene $bla_{\text{CTX-M-1}}$ in *Salmonella enterica* isolates from poultry and humans in France, 2003 to 2008. Antimicrob Agents Chemother. 2010;54:4484-6.

80. Wittum TE, Mollenkopf DF, Erdman MM. Detection of *Salmonella enterica* isolates producing CTX-M cephalosporinase in U.S. livestock populations. Appl Environ Microbiol. 2012;78:7487-91.

81. Freire Martín I, AbuOun M, Reichel R, La Ragione RM, Woodward MJ. Sequence analysis of a CTX-M-1 IncI1 plasmid found in *Salmonella* 4,5,12:I:-, *Escherichia coli* and *Klebsiella pneumoniae* on a UK pig farm. J Antimicrob Chemother. 2014;69:2098-101.

Fig. 1 ESBL variants that are found in identical *E.coli* clones and/or encoded on identical plasmids originating from fecal samples of farmers and of livestock, and ESBLs that are disseminated from farms to the farm environment via airing systems, gastrointestinal waste, or insects